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## 2,3,7,8-Dibenzo-*p*-dioxins in Mined Clay Products from the United States: Evidence for Possible Natural Origin

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Ball clay was the source of dioxin contamination discovered in selected chickens analyzed as part of a joint U.S. Department of Agriculture/U.S. Environmental Protection Agency national survey of the U.S. poultry supply conducted in 1997. The affected animals, which had been raised in the southern United States, represented approximately 5% of the national poultry production. All of these chickens and other animal food sources (i.e., farm-raised catfish), similarly contaminated, were fed a diet of animal feed containing ball clay as an anti-caking additive. The clay was mined in northwestern Mississippi within a geological formation referred to as the Mississippi Embayment. Individual raw and processed ball clay samples were analyzed for the presence of the 2,3,7,8-PCDDs/-PCDFs. The average toxic equivalents (TEQs) for the raw and processed samples were 1513 and 966 ppt dw, respectively. Other mined clay-based products used in animal feeds revealed lower TEQs. All of the products exhibited either an absence of detectable concentrations of 2,3,7,8-PCDFs or concentrations 2–3 orders of magnitude lower than the PCDDs. The isomer distribution, specific isomer identification, and congener profile of the PCDDs in the clay were established and compared to known sources of dioxin contamination. Several unique features of this isomer distribution are characteristic of the clays and are distinguishable from those of other known sources. These characteristics found in prehistoric clay deposits in the United States and Germany have led to speculation attributing their presence to natural geologic processes.

### Introduction

Several recent studies have found elevated levels of 2,3,7,8-chlorine substituted polychlorinated dibenzo-*p*-dioxins (PCDDs) in fryer chickens and other animal products grown in the United States resulting from the use of contaminated

animal feed (1, 2). Subsequent investigations performed at the USEPA/OPP Environmental Chemistry Laboratory into possible sources of the PCDDs in the feed (e.g., soybeans, lipid additives, rice hull ash, ball clay) demonstrated conclusively that the ball clay was the ultimate source of the PCDDs found in the chickens and other animal food products fed a diet containing the clay additive in their feed. The dried processed clay was used as an anti-caking and/or pelleting aid in nonmedicated feeds at levels not to exceed 2.5% in finished feed. The use of this particular clay in animal feed has been discontinued (3). The concentration and PCDD isomer distribution in this raw ball clay were strongly correlated with the concentration and isomer patterns of the PCDDs in the processed clay, the amount of clay added to the animal feeds, and the subsequent concentrations of the PCDDs in the fryer chickens. These relationships have also been established in other food products by other researchers (4–6).

Raw ball clay samples were obtained directly from the mine known to be in the area from which the processed clay added to the feed had been mined. The mine located in Sledge, MS, is part of a geological formation referred to as the Mississippi Embayment, which extends over approximately 45 000 mi<sup>2</sup> (7). Ball clay consists predominately of poorly defined crystalline kaolinite. This clay is of the Zilpa clay that are in sedimentary beds laid down during the early to middle Eocene Epoch, which occurred approximately 40–45 million years ago and is the second geologic epoch of the Tertiary period marked by the emergence of terrestrial mammals and present-day vegetation (8). Although the geomorphology of ball clay is not well understood, a defining feature is that it contains a significant amount of carbonaceous material. Over one million metric tons of ball clay was processed in the United States in 1998 and is used in a variety of ceramic products, including floor and wall tile, sanitary ware, and pottery (9). Whether the dioxins in the clay are destroyed or volatilized and released into the environment during the firing process has yet to be established.

In a subsequent joint project between the U.S. EPA and the U.S. FDA, 15 other processed mined products used as animal feed additives (e.g., bentonite, sodium bentonite, montmorillonite, limestone, attapulgite) were prepared and analyzed at this laboratory by high-resolution gas chromatography/high-resolution mass spectrometry (HRGC/HRMS) for the presence of 2,3,7,8-PCDDs/PCDFs and the extracts subsequently reanalyzed by quadrupole ion storage mass spectrometry/mass spectrometry (QISMS/MS) (10).

The concentrations of the PCDDs/PCDFs in eight samples of raw ball clay and 19 various processed mine products, their associated TEQs, and the congener profiles and isomer distributions of the 2,3,7,8-PCDDs in the ball clay from the northern Mississippi mine are presented in this report. The significance and usefulness of the congener profiles and isomer distributions as a means to characterize sources/origin are also discussed.

### Experimental Section

**Sampling.** Raw ball clay occurs as lenses interstratified with sand, silt, and lignite and is conventionally mined by surface strip mining techniques involving the use of heavy earth moving and excavation equipment. Eight individual raw ball clay samples were collected from an open mining pit at a depth of 50–60 ft by boring horizontally into the wall adjacent to the excavated area to obtain clay not previously exposed

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**TABLE 1. Concentration of 2,3,7,8-Cl Substituted PCDDs in Raw Ball Clay\***

congener	average	% RSD	median	min	max	WHO-TEF	TEQ
2,3,7,8-TCDD	711	54.6	617	253	1259	1.00	711
1,2,3,7,8-PeCDD	508	43.3	492	254	924	1.00	508
1,2,3,4,7,8-HxCDD	131	31.2	134	62	193	0.10	13
1,2,3,6,7,8-HxCDD	456	35.3	421	254	752	0.10	46
1,2,3,7,8,9-HxCDD	2093	41.1	1880	1252	3683	0.10	209
1,2,3,4,6,7,8-HpCDD	2383	32.5	2073	1493	3346	0.010	24
OCDD	20640	82.4	4099	8076	58766	0.0001	2
total TEQ							1513

\* ppt, dw,  $n = 8$ .

to the atmosphere. The clay was transferred to precleaned glass jars and stored until analysis.

Four associated processed ball clay samples were sent to the laboratory as subsamples of larger bulk quantities in prepackaged containers that were suitable for commercial sale. The raw ball clay is processed by means of a series of milling stages. The first stage involves the shredding of the lump ball clay to a uniform size using flatbed shredders. The clay is then fed into impact mills where hot air (120 °C) from a furnace impinges on the clay and is dried and pulverized to form a product that is referred to as airfloated ball clay. This clay is packaged and constitutes the "processed" ball clay. Upon receipt, these samples were transferred to precleaned jars and stored until analysis.

Fourteen processed mined clay products and one limestone were sampled by the U.S. FDA investigators at a variety of commercial processing locations in the United States and transferred to precleaned jars and shipped to the lab where they were stored until analysis. All of the ball clay samples were analyzed along with associated processed clay samples for the presence of PCDDs/PCDFs.

**Analysis.** The procedures used to analyze the clay and mined clay samples for PCDDs/PCDFs were described in Ferrario et al. (11). These procedures were based on a modified version of U.S. EPA Method 1613 (12). Prior to sample analyses, the mass spectrometer was tuned to a minimum resolution of 10 000 and calibrated. The linearity of the calibration curve was verified by analyzing Calibration Standard 2 (200 fg/ $\mu$ L TCDD), and the mass chromatogram was examined to ensure that all the 2,3,7,8-Cl substituted congeners were clearly separated. If the S/N ratios were  $\geq 10$ , the ion abundance ratios were within  $\pm 20\%$  of theoretical, and the response factors and isomer separations were within specified limits, then sample analyses proceeded. Corrective action was initiated if specified control limits were exceeded. Peak identification criteria were as follows: S/N  $\geq 3.5$ ; the isotope ratio of the two characteristic ions for each congener class within 20% of the theoretical value; the peak maxima for the molecular ion cluster ions coincide within 2 s; and native analytes elute within 3 s of their corresponding  $^{13}\text{C}$ -labeled analogues. Method blanks were examined for the presence of interfering background, and no value for any analyte was reported unless it was twice the level of the blank after background subtraction. The blanks in this study were free from interferences and demonstrated no cross contamination between samples. The results from individual sample sets were reviewed by the Quality Assurance Officer (QAO) to ensure compliance.

Prior to the initiation of sample preparation and analyses, a Quality Assurance Project Plan (QAPP) was prepared and submitted to an independent QAO for review and approval. The QAPP addressed each of the following critical elements: project description, organizational responsibilities, data quality objectives for measurements, sampling procedures, data reduction, validation and reporting, internal QA checks, audits, preventive maintenance, corrective actions, and QA reports to management.

Many of the details regarding sample preparation procedures, analytical techniques, and quality control strategies parallel those defined in U.S. EPA Method 1613. Specific studies providing additional details regarding the analytical procedures and quality control thresholds and/or specific changes to Method 1613 have been previously described by Ferrario et al. (13, 14).

Sample sets consisted of one method blank, one laboratory-spiked control sample or duplicate, and between 6 and 9 samples. After removal from storage, samples were tracked through preparation, extraction, cleanup, and analyses. Briefly the procedure was as follows:

Approximately 2 g of clay was ground to a powder mixed with sodium sulfate, fortified with a mixture containing 100 pg of each of the 17  $^{13}\text{C}$ -labeled 2,3,7,8-Cl substituted PCDDs/PCDFs recovery surrogates (Cambridge Isotopes) and Soxhlet extracted with benzene. The extracts were cleaned using a combination of acidified and basic silica gel, alumina, and carbon column chromatography. The final extracts were spiked with  $^{13}\text{C}$  internal standards, and the volume was adjusted to 20  $\mu$ L final volume prior to analysis. A 1–2- $\mu$ L subsample of the final extract was analyzed using a 60-m DB-5MS column by HRGC/HRMS. The KRATOS Concept mass spectrometer was operated in the mass drift correction mode, and the native analyte concentrations were determined by isotope dilution. The samples were analyzed for the 17 2,3,7,8-Cl substituted PCDDs/PCDFs that have been assigned toxic equivalency factors and the homologue totals.

To determine the isomer distributions and specific congener identifications, samples were analyzed using both J&W Scientific DB-5 and DB-5MS columns (60 m  $\times$  0.32 mm, 0.25  $\mu$ m film thickness). Isomer identifications were initially established using a DB-5 column employing the use of authentic standards (Cambridge Isotopes, Inc), if available, and by the use of standard chromatographic techniques employing relative retention times (RRTs). Identifications not made using authentic standards were determined by generating RRTs and comparing them to those derived by Ryan et al. (15) under the chromatographic conditions described therein. The agreement of the RRTs between the two laboratories was excellent.

The limit of detection (LOD):limit of quantification (LOQ) for the PCDDs/PCDFs in the 2-g clay samples were 0.5:1 pg/g (ppt), dw for the tetras; 1.0:2.0 ppt for the pentas, the hexas, and the heptas; and 5.0/10.0 ppt for the octas.

## Results

The results of the analyses of eight individual samples of the raw ball clay are presented in Table 1. The concentrations are presented in ppt dw, and the individual toxic equivalents (TEQs) and total TEQs are also provided calculated using the World Health Organization (WHO) recommended toxic equivalent factors (WHO-TEFs) (16). As is evident from Table 1, the average concentrations of all of the 2,3,7,8-PCDDs exceeded 100 ppt. OCDD is found at the highest concentration in all of the samples followed by either the 1,2,3,4,6,7,8-

**TABLE 2. Concentrations of 2,3,7,8-Cl Substituted PCDDs in Processed Ball Clay<sup>a</sup>**

congener	average	% RSD	median	min	max	WHO-TEF	TEQ
2,3,7,8-TCDD	48	52.4	38	31	85	1.00	48
1,2,3,7,8-PeCDD	432	8.3	434	395	464	1.00	432
1,2,3,4,7,8-HxCDD	593	13.0	604	500	664	0.10	59
1,2,3,6,7,8-HxCDD	738	10.0	755	635	807	0.10	74
1,2,3,7,8,9-HxCDD	2028	16.0	2010	1683	2410	0.10	203
1,2,3,4,6,7,8-HpCDD	14041	8.8	13980	12836	15367	0.010	140
OCDD	205663	45.2	211244	90810	309353	0.0001	21
total TEQ							977

<sup>a</sup> ppt, dw, *n* = 4.

HpCDD or, surprisingly, in two of the samples by the 1,2,3,7,8,9-HxCDD. The maximum OCDD concentration is approximately 59 ppb. The most toxic tetra and penta congeners are present at unusually high concentrations in all of the samples with average concentrations of 711 and 508 ppt for 2,3,7,8-TCDD and 1,2,3,7,8-PeCDD, respectively. The other congeners have a 31–82% RSD.

Other interesting features associated with these samples are the much lower concentrations, and in some samples, the absence of the 2,3,7,8-PCDFs at comparable detection limit concentrations (LOD = 1 ppt) and the presence of the 1,2,3,7,8,9-HxCDD at higher concentrations than the other 2,3,7,8-HxCDD isomers. The average percent distribution among the three individual 2,3,7,8-hexa isomers is 5:17:78. These features are characteristic for all the raw ball clay samples analyzed. The average total TEQ for the raw ball clay is 1.5 ppb dw with the TEQ for 2,3,7,8-TCDD comprising the largest percent at 47%, followed by 1,2,3,7,8-PeCDD at 34%. As expected the OCDD, even though present at the highest concentration, contributes less than 1% of the total TEQ due to its relatively small WHO-TEF. In comparison, the range of dioxins in surface soil samples varies between rural and urban areas from about 1 to 20 ppt TEQ (17).

The results of the analyses of the four associated processed clay products are presented in Table 2. As with the raw ball clay, OCDD is present at the highest average concentration with its absolute and relative concentration being much higher in the processed samples than in the raw ball clay with an average OCDD concentration exceeding 200 ppb. 1,2,3,4,6,7,8-HpCDD was next in concentration followed, in all cases, by the 1,2,3,7,8,9-HxCDD. The relationship between the HxCDD congeners as described above was maintained in all the processed clay samples. It is interesting to note that although the PeCDD concentrations in both the raw ball clay and the associated processed clay were approximately equal with average concentration of 500 and 430 ppt, respectively, there was a dramatic reduction in the concentration of the TCDD in the processed clay. The average concentration of the TCDD in the raw ball clay was approximately 700 ppt but only about 50 ppt in the processed clay. Whether the changes in the profiles between raw and processed clay are due to the processing and whether these changes reflect the loss and/or formation of specific congeners remains to be established.

The average homologue totals are presented in Table 3, and as is evident, the furans concentrations, while detectable, are drastically lower than the dioxins in each homologue group, and the relative contribution of each individual group to the total is markedly different between raw and processed clay. In fact, several 2,3,7,8-PCDFs were detected in some of the samples at a detection limit of approximately 1.0 ppt, but due to the small sample size used for most of the samples, these levels were close to method blank values. As a comparison the homologue totals from "background" urban and rural locations have been added to the table.

The results from the analyses of the other mined clay products containing detectable levels of PCDDs are presented

**TABLE 3. Average Concentrations of Total Congener Groups for Raw and Processed Ball Clay<sup>a</sup> Compared to Background Soils**

total congeners	raw ball clay	processed ball clay	urban background	background ( <i>n</i> = 3)
TCDD	3729	2425	4	1
TCDF	6	11	11	2
PeCDD	4798	7378	12	0.3
PeCDF	2	1	24	2
HxCDD	6609	21034	22	5
HxCDF	6	12	19	3
HpCDD	6194	30420	60	38
HpCDF	9	55	19	12
OCDD	11222	211244	345	161
OCDF	11	23	9	11
ΣD/F	957	2672	5	7

<sup>a</sup> ppt, dw, *n* = 2.

in Table 4. A total of 15 mined products used in animal feeds were analyzed. Six samples were virtually free from detectable levels of PCDDs/PCDFs including OCDD. These included five bentonites or sodium bentonites from Wyoming, South Dakota, or the New England states and the attapulgite from Florida. The congener found at the highest concentration was OCDD followed without exception by the HpCDD and then the 1,2,3,7,8,9-HxCDD which, as in all the mined clay products analyzed, is present at higher concentrations than the other two toxic hexa isomers. It is interesting that this relationship among the toxic hexa congeners is seen in all the mined clay products analyzed not just in the ball clay. As is evident from the tables the TCDD concentrations, when detected at all, were significantly lower than those determined for the ball clay. The total TEQs ranged from less than 0.5 ppt in the limestone to as high as 22.4 ppt in one of the bentonites. For those samples with the higher TEQs, the primary dioxin congener in terms of its contribution to the total TEQ was the 1,2,3,7,8-PeCDD. It is interesting to note that all of the mined samples with significant concentrations of PCDD/PCDFs are located in the southeastern portion of the United States.

The isomer distribution of the tetra-, penta-, and hexachlorinated homologues of the raw ball clay are presented in Figure 1. The hepta- and octa-chlorinated homologues profiles are unremarkable in that the heptas contain only two possible isomers and the octa contains only one, and thus the chromatograms are not displayed. The concentration of the 1,2,3,4,6,7,9-HpCDD is approximately 1.5 times greater than that of the 1,2,3,4,6,7,8-HpCDD and elutes first on both columns.

## Discussion

As is evident in Figure 1, the tetra isomer distribution is dominated by the 2,3,7,8-, 1,2,7,8-, 1,2,6,7-, and 1,2,8,9-congeners. This is in marked contrast to the distribution exhibited by a "typical" air sample, a suspected primary source from aerial deposition (Figure 2), in which these isomers are minor components and the profiles are domi-

**TABLE 4. Concentration of 2,3,7,8-Cl Substituted PCDDs in Mined Clay Product\***

product	limestone	Al—Ca—Si	bulk clay	montmorillonite			ground clay	bentonite	bentonite
location	TN	TX	GA	GA	MS	MS	GA	MS	MS
2,3,7,8-TCDD	nd	nd	nd	0.25	nd	nd	0.72	nd	1.55
1,2,3,7,8-PeCDD	nd	0.41	0.53	nd	0.79	2.14	3.51	1.47	8.67
1,2,3,4,7,8-HxCDD	nd	nd	0.64	1.04	1.02	2.47	5.74	2.22	12.2
1,2,3,6,7,8-HxCDD	0.74	0.30	0.66	1.18	1.54	2.58	6.23	2.53	15.6
1,2,3,7,8,9-HxCDD	2.41	0.62	1.71	1.99	2.15	3.09	7.87	3.07	20.4
1,2,3,4,6,7,8-HpCDD	9.77	2.38	13.0	16.6	23.2	84.9	179.9	84.6	361.5
OCDD	145	3.98	82.2	70.0	1325	10052	17281	8435	37519
total TEQ	0.43	0.52	0.97	0.84	1.47	4.81	9.75	3.94	22.4
total TCDD	1.33	nd	1.07	1.59	2.26	3.18	26.74	9.25	69.4
total PeCDD	nd	nd	nd	nd	0.79	10.22	28.69	13.31	130
total HxCDD	14.00	0.92	19.02	nd	26.21	60.0	138	63.33	323
total HpCDD	19.38	9.88	49.56	24.58	65.47	234	436	200	948
total OCDD	145	3.98	82.20	45.67	1325	nd	17281	8435	37519
total TCDF	nd	nd	0.05	0.17	nd	nd	0.38	nd	2.6
total PeCDF	nd	nd	nd	nd	nd	nd	nd	nd	0.86
total HxCDF	nd	nd	nd	nd	nd	nd	0.13	nd	0.90
total HpCDF	0.19	nd	nd	nd	nd	0.27	0.36	0.35	0.89
total OCDF	nd	nd	nd	nd	nd	nd	nd	11.86	nd

\* ppt, dw.

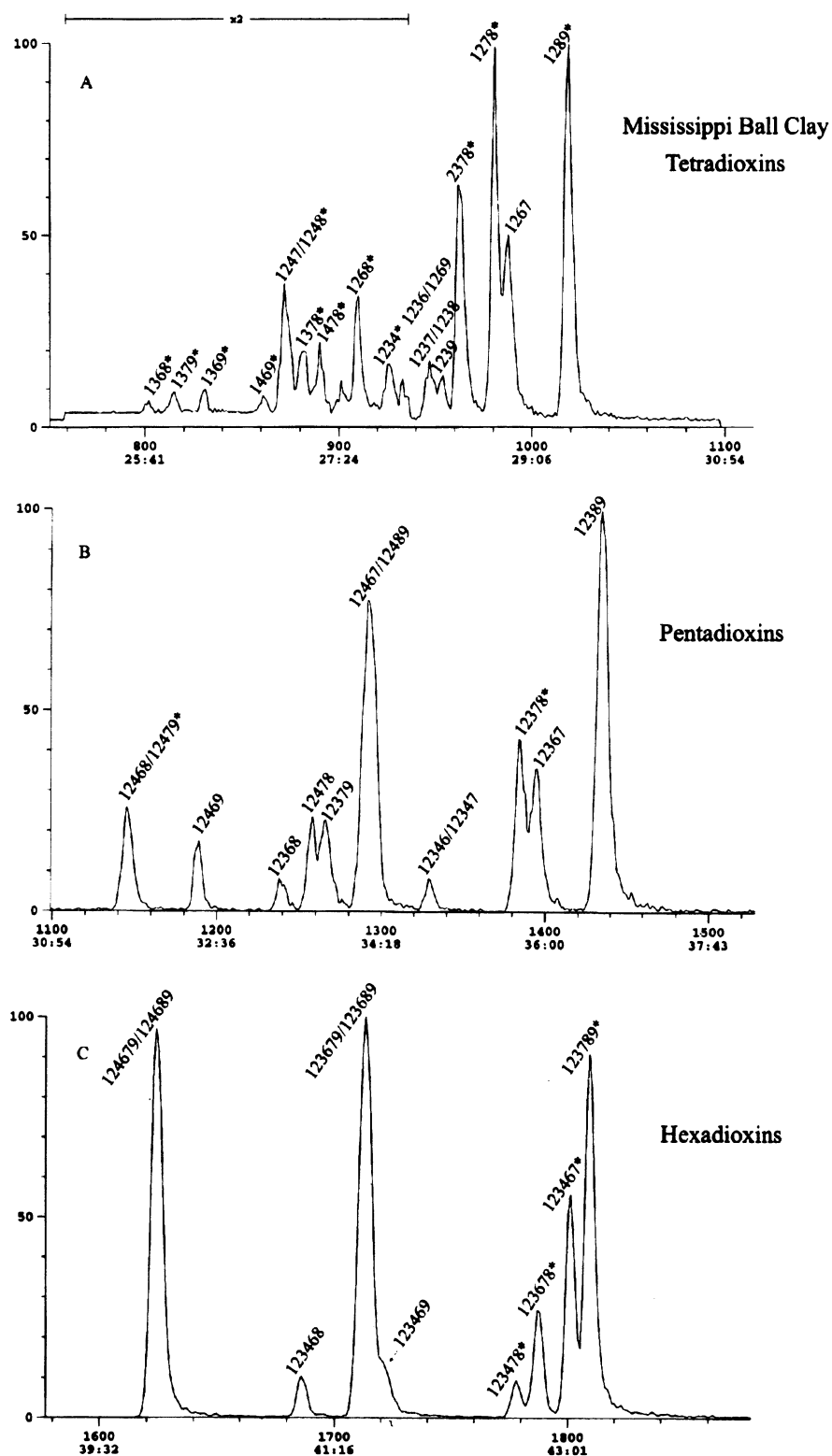
nated by the 1,3,6,8-, 1,3,7,9-, 1,3,6,9-, and 1,2,4,7-/1,2,4,8- (unresolved) congener pair. Other interesting features are the absence of any of the 2,3,7,8-Cl substituted furans at comparable concentrations and the presence of 1,2,3,7,8,9-HxCDD at higher levels than the other 2,3,7,8-HxCDD isomers. This is unusual since, in most cases, the 1,2,3,6,7,8 is the dominant congener among the toxic hexa isomers, especially in biological media (18–21). This situation regarding the hexa isomers has also been observed by Rappe et al. (21) and seems to be a general trend for most food samples from around the world. However, this specific HxCDD distribution has been identified in Mississippi sediments without an anthropogenic input by Rappe et al. (22). It is interesting to note that the tetra isomer distribution reported in catfish feed was dominated by the same congener pattern seen here and in chicken feed and both have been shown to have resulted from the use of ball clay in the soybean meal component of the feed. Moreover, the distinctive features of the 2,3,7,8-congener profile seen in the ball clay were also retained by chicken and catfish (2, 4, 6, 21). This demonstrates the conservative, stable, and reproducible nature of these isomer patterns and indicates their value as reliable indicators of their source and/or origin. However, there are others that have described instances where they believe the congener profile is not indicative of the original source (17).

**Anthropogenic Sources.** Cleverly et al. (23) have reported on the congener profiles that are typical of known anthropogenic sources of dioxin-like compounds in the United States. These analyses was used as a basis for comparison to the profile of the raw ball clay (see Figure 3). The congener pattern characteristic of combustion sources of dioxin-like compound differ significantly from the ball clay profile in that all 2,3,7,8-Cl substituted PCDD and PCDF congeners are measured in stack emissions. In combustion source emissions, 2,3,7,8-TCDD is usually 0.1–1.0% of total PCDD/PCDF mass emitted whereas 2,3,7,8-TCDD is approximately 5% of total mass of dioxins present in the clay. As with the ball clay, the most prevalent 2,3,7,8-Cl substituted PCDD congeners in most incinerator emissions are as follows: OCDD, 1,2,3,4,6,7,8-HpCDD. However, combustion emissions contain appreciable amounts of PCDFs of which the 1,2,3,4,6,7,8-HpCDF, OCDF, 1,2,3,4,7,8-HxCDF, 2,3,7,8-TCDF, and 2,3,4,6,7,8-HxCDF congeners dominate. The congener profile of 2,4-D salts and esters seems to mimic a combustion source profile in the number of congeners represented and in the minimal amount of 2,3,7,8-TCDD relative to all 2,3,7,8-Cl substituted congeners. Nevertheless, unlike the combustion source profile, the 1,2,3,7,8-PeCDD and the 1,2,3,4,6,7,8-

HpCDF constitute major fractions of total PCDD/PCDF contamination present in 2,4-D. The congener profile of technical grade pentachlorophenol (PCP) is clearly dominated by OCDD and 1,2,3,4,6,7,8-HpCDD. However, only trace amounts of 2,3,7,8-TCDD are detected in PCP, and 1,2,3,4,6,7,8-HpCDF and OCDF constitute roughly 15% of typical formulations. Metal smelting and refining processes, such as secondary aluminum, copper, and lead smelting, also have all the 2,3,7,8-Cl substituted congeners of PCDDs/PCDFs in stack emissions. In secondary aluminum smelting, 2,3,7,8-TCDD is less than 0.1% of total PCDDs/PCDFs whereas PeCDF is nearly 25% of total emissions of dioxin-like compounds, and the PCDD/PCDF ratio is <1. Secondary copper operations show a similar pattern of PCDD/PCDF emissions but with five compounds dominating emissions: 1,2,3,4,7,8-HxCDF; 1,2,3,6,7,8-HxCDF; 1,2,3,4,6,7,8-HpCDF; OCDF, OCDD, and 1,2,3,4,6,7,8-HpCDD. In iron ore sintering, the dominant congener in emissions of 2,3,7,8-Cl substituted compounds is 2,3,7,8-TCDF. The combustion of wood generates a congener profile not unlike that of waste incinerator (i.e., the ratio of PCDD/PCDF < 1), and all laterally substituted congeners can be detected in emissions. In the preparation of wood pulp needed in the production of paper, bark is stripped from the exterior of newly harvested trees. This produces a woodwaste that is usually combusted in a wood-waste hog-type incinerator at the paper mill. The combustion of tree bark produces a congener profile in which the PCDD/PCDF ratio is > 1, showing only minimal and barely detectable levels of PCDFs in the smoke. The dominant congener in tree bark combustion emissions is OCDD (>30% total PCDD/PCDF mass), followed by 1,2,3,4,6,7,8-HpCDD and 1,2,3,7,8,9-HxCDD.

A comparison of the characteristic profile of the ball clay to those of known anthropogenic sources from the U.S. EPA source inventory has been undertaken, and none of those examined match the features characteristic of these clays (24). The source of the dioxins found in the original ball clay has yet to be established. However, the characteristic features of the clay together with the fact that the geologic formations in which the samples were taken are ancient suggest a natural origin for the dioxins.

**Natural Sources.** The significance of the presence of dioxins in historical samples (25–27) and the relevance of the information derived from studies in temporal trends (28–30) has been reviewed by other investigators, and those interested are directed to these reviews. Briefly, these studies focus attention on the contribution of known and/or suspected dioxins sources to the background levels seen in



**FIGURE 1.** Isomer distribution of the tetra- (A), penta- (B), and hexa-chlorinated dioxins (C) in ball clay from Sledge, MS. An asterisk (\*) indicates using authentic analytical STD.

various samples collected over time in an attempt to discern the relative contribution of contemporary sources of an anthropogenic origin from any natural background that could have been derived from natural historical processes (e.g., forest fires). The elevated dioxin concentrations and isomer pattern seen in the clays appear to be fundamentally different from those described previously for known sources and may represent another example of the natural formation of these compounds in ancient sediments.

Over 20 years ago, Bumb et al. (31) argued that the ubiquity of dioxins in the environment was due to natural phenomena, most importantly the trace chemistry of fire. The natural origin or production of various dioxin congeners by natural processes has been suggested and demonstrated by a number of investigators. Lamparski et al. (32) reported that hexa-, hepta-, and octadioxin congeners in sewage sludge sealed in an ampule from 1933 matched the pattern found in sewage sludge from the 1980s. Since the 1933 sample predated the

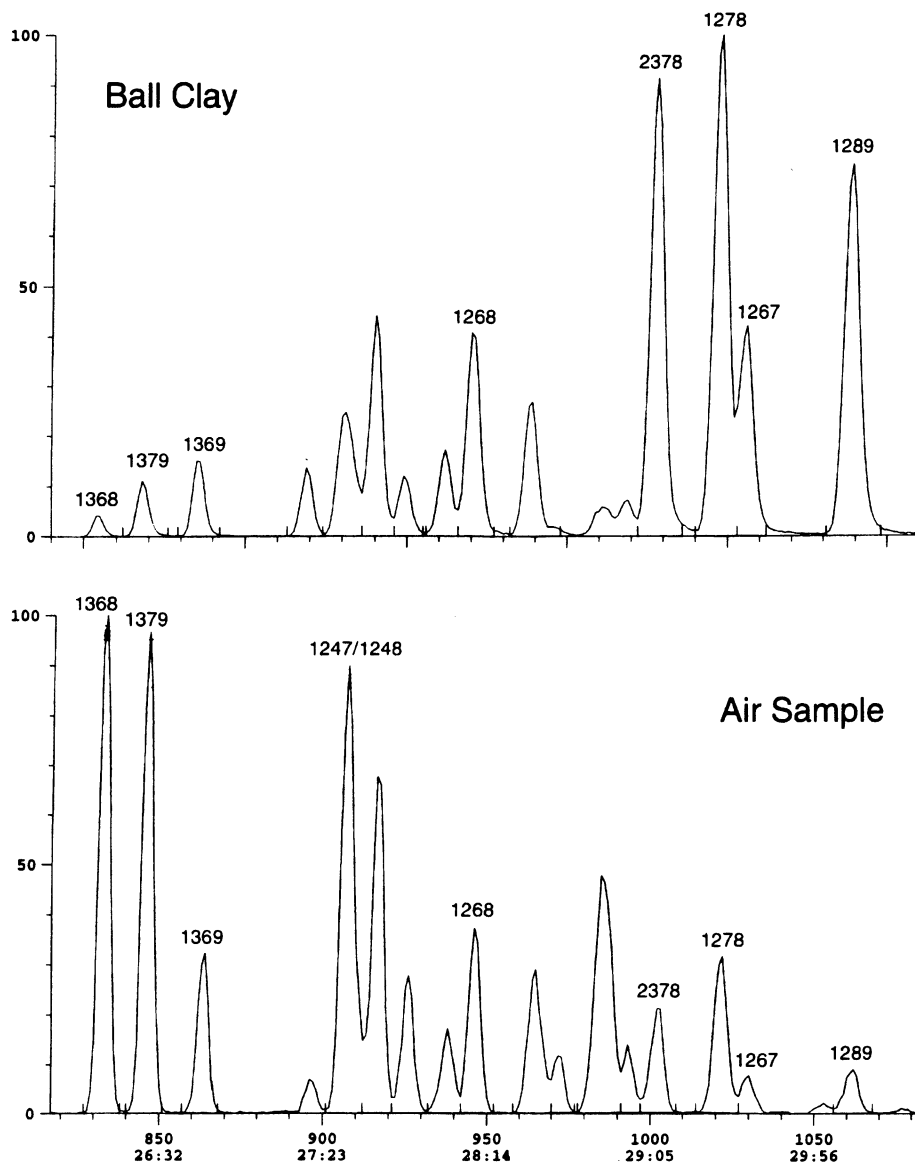


FIGURE 2. Tetrachlorodibenzo-*p*-dioxin isomer distributions. (A) Ball clay; (B) air sample.

commercial production of pentachlorophenol [PCP] (a likely source for these congeners), a natural chlorination process was proposed to account for their presence. Similarly, Svenson et al. (33), Oberg et al. (34), Rappe et al. (35), and Silk et al. (36) all reported enzyme-mediated dioxin formation from chlorophenols. The biosynthesis of dioxins in cows has also been suggested by Fries et al. (37). Additionally, Rappe concluded from studies done in southern Mississippi that the characteristic dioxin profiles with their high D/F ratios seen in cores from naturally formed and manmade lakes dated before any possible anthropogenic sources and with no known anthropogenic input confirm a natural source. Moreover, he concludes that the hexadioxin pattern seen precludes PCP as the likely source and the high hepta- and octadioxins cannot be explained by aerial deposition (38).

The presence and natural production of a wide variety of halogenated organics including an assortment of chlorinated phenols by ancient species of blue green alga, fungi, and numerous other prehistoric organisms is described in detail by Gribble (39). More recently, Hoekstra et al. (40) has demonstrated the natural formation of chlorinated phenols and chlorinated dioxins/furans in the soil of a Douglas fir forest and has proposed a mechanism to account for their

formation. Moreover, he illustrates the importance and usefulness of identifying the specific congeners present as a means of gaining insight into the reaction mechanisms responsible for their formation. It is interesting that Hoekstra et al. focus attention on the natural production of 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD, and 1,2,3,7,8,9-HxCDD, which are also dominant congeners in the clay invariably found in the clay from the Mississippi Embayment. The accumulation of these naturally formed chlorinated materials in the clay deposits over the eons could conceivably have supplied the precursor materials for the formation of the dioxins seen in the clays.

It is intriguing that the dioxin profiles associated with the chick edema syndrome recently presented by Hayward et al. (6), although distinguishable from the clays, share in common with the ball clays the predominance of the 2,3,7,8-, 1,2,7,8-, 1,2,6,7-, and the 1,2,8,9-TCDD congeners; the dominance of the 1,2,3,7,8,9-HxCDD among the toxic hexa isomers; and the absence of PCDFs. This fat was reportedly derived from cowhide treated with several chlorinated phenols and subsequently hydrolyzed and distilled (41). The production of dioxins from chlorophenols is well-documented, and these shared characteristics invite speculation that the dioxins in the clays could have possibly been derived from naturally

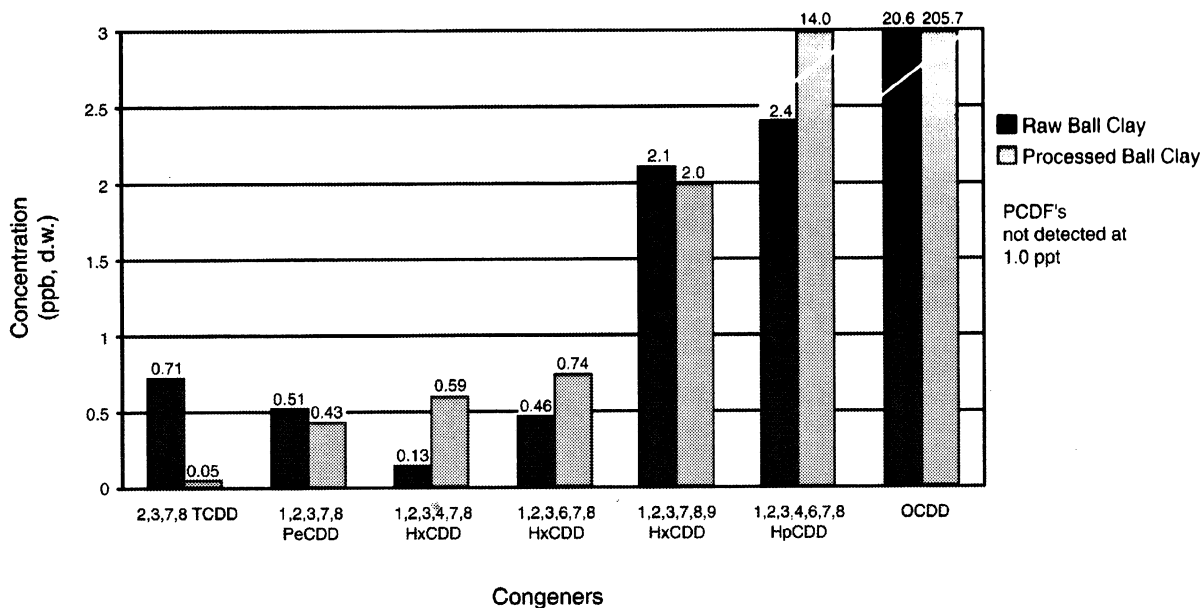


FIGURE 3. Congener profile and average concentrations of PCDDs in raw and processed ball clay.

produced chlorophenols by as a yet unexplained mechanism. However, the possibility that the clays have somehow functioned as a sink for the accumulation of dioxins that were once contaminants of anthropogenic chlorinated phenols has not been ruled out.

The absence of the furans at comparable concentrations to the dioxins is another interesting feature of the ball clay. The available evidence indicates that PCDDs/PCDFs, particularly the tetra and higher chlorinated congeners, are extremely stable under most environmental conditions but are subject to microbial degradation in anaerobic environments. On the basis of information pertaining to the environmental fate of organic chemicals, the half-lives of the 2,3,7,8-Cl substituted dioxins and furans are approximately equal in the same environmental compartment and progressively increase from air to water, soil, and sediment with half-lives increasing with increased chlorination (42). However, more recent studies have shown that, under extreme hydrothermal conditions, PCDDs/PCDFs are destroyed in an aqueous alkaline solution beginning at 200 °C and reaching 99% at 300 °C. These decomposition rates increase with increasing pH. Under these conditions, furans are destroyed faster than dioxins, higher chlorinated PCDDs/PCDFs are destroyed faster than lower chlorinated species, and congeners having an odd number of chlorines are more susceptible (43). Without additional studies specifically addressing the differential degradation of dioxins/furans in ball clay, the absence of furans in the clay remains unexplained. The absence of the furans at comparable concentrations may also provide some insight into a possible formation mechanism for the dioxins found in the clays. As mentioned earlier in this paper and observed by others, the absence of furans in the ball clay at comparable concentrations as reflected by the elevated D/F ratio do not seem to match other known sources. It is well-known that the D/F ratio seen in a typical air sample presumed to have originated from combustion sources is much closer to unity with a dominance of furans for the tetra and penta homologues depending on the combustion conditions. Additionally, the tetradoxin isomer distribution as noted earlier is unlike the "typical" tetradoxin distribution seen in air samples, which reflects and is indicative of combustion sources (44). The virtual absence of furans in the clays and the presence of a tetradoxin profile uncharacteristic of air samples or combustion sources implies a formation mechanism other than

those proposed to occur during the combustion process to account for the formation of the dioxins in clay. Moreover, assuming that the particular tetra isomers present in the clay are indicative of the thermodynamics and/or starting materials present during their formation, a critical evaluation of the isomers present may provide some insight into the formation process itself.

Another ball clay sample originating from a mine in Kentucky was also found to contain elevated PCDD concentrations (3.5 ppb) comparable to the clay mined in Mississippi and shares the same features noted for the isomer distribution and the congener profiles. This clay is of the same age and sedimentary origin as that from Mississippi and is remarkably similar in mineral content (7). Recently, the presence of the 2,3,7,8-PCDDs have been discovered in kaolinitic clay mined in Germany (45), resulting in the European Union Directive establishing as an emergency measure a provisional maximum dioxin limit of 0.50 ng/kg for these clays that can be used as an additive in animal feed. No anthropogenic source was suspected, and it was speculated that the dioxins were the result of some geologic process. In addition, the German clay also shares the characteristics of the Mississippi ball clay notably, a remarkably similar congener profile with an absence of furans at comparable concentrations and the predominance of the 1,2,3,7,8,9-HxCDD among the toxic hexa congeners. It would be instructive to determine if the clay deposits in Germany are also located in sedimentary beds of a similar geologic epoch as those found in the United States. The widespread distribution of the dioxins in mined clay products from several different locations in the United States and in Germany that share the same characteristic isomeric features argues for at least a common origin.

Of course, the possibility that the clays could be acting as sinks for the accumulation of dioxins from anthropogenic sources cannot be dismissed. Samples reportedly taken at mine locations outside and above the level of the clay lenses and from areas outside the mine itself showed ppb levels of 2,3,7,8-TCDD. Several of these locations are known to have been treated with herbicides (46). Nonetheless, these recent findings of elevated dioxin concentrations in clays from deposits reported to be million of years old in disparate regions in the United States with distinct unprecedented isomer patterns that reportedly match those originating from ancient deposits in Germany currently argues for some



common natural geologic mechanism to account for their origin. To date, no definitive experimental evidence has been brought forward either to account for the presence of the dioxins from known anthropogenic sources or to explain the selective chemical synthesis of PCDDs under the conditions inherent to the formation of clays some 40 million years ago. A systematic evaluation of the distribution of dioxins in these clay deposits and the surrounding area and a consideration of the specific isomers present as indicators of the formation mechanism and/or source may provide the information needed to develop testable hypotheses in order to identify the process or processes responsible for their occurrence.

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## Nomenclature

PCDDs	polychlorinated dibenzo- <i>p</i> -dioxins
PCDFs	polychlorinated dibenzo- <i>p</i> -furans
TCDD	tetrachlorinated dibenzo- <i>p</i> -dioxin
PeCDD	pentachlorinated dibenzo- <i>p</i> -dioxin
HxCDD	hexachlorinated dibenzo- <i>p</i> -dioxin
HpCDD	heptachlorinated dibenzo- <i>p</i> -dioxin
OCDD	octachlorinated dibenzo- <i>p</i> -dioxin
ppt	parts per trillion
dw	dry weight
WHO-TEF	World Health Organization toxic equivalent factors
TEQ	toxic equivalent

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